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Improved drought tolerance in *Festuca ovina* L. using plant growth promoting bacteria

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Abstract: Numerous ecological factors influence a plant's ability to live and grow, in which dryness is a substantial constraint on plant growth in arid and semi-arid areas. In response to a specific environmental stress, plants can use the most effective bacteria to support and facilitate their growth and development. Today, plant growth promoting rhizobacteria (PGPR) is widely used to reduce drought stress on plant growth. In this study, the effects of drought on *Festuca ovina* L. germination, growth, and nutrient absorption were investigated using PGPR in a factorial test with a completely random design under four water regimes. Soil water content was kept at 100% FC (field capacity), 70% FC (FC), 50% FC, and 30% FC. The treatments were inoculated with *Azotobacter vinelandii*, *Pantoea agglomerans*+*Pseudomonas putida*, and a mixture of bio-fertilizers. Results showed that the effects of drought stress were significantly reduced (P<0.05) when *A. vinelandii* and *P. agglomerans*+*P. putida* were used separately, however, the combined treatment of bio-fertilizers had a greater influence on seed germination than the single application. *P. agglomerans*+*P. putida* under 30% FC condition resulted in higher increases in stem, root length, and plant dry biomass. The highest uptake of nutrients was observed for the combined treatment of bio-fertilizers under 30% FC condition. Therefore, the use of *A. vinelandii* and *P. agglomerans*+*P. putida*, applied separately or combined, increased tolerance to drought stress in *F. ovina* by increased germination indices, dry weight, stem length, and root length. Because of the beneficial effects of PGPR on the growth characteristics of plants under drought conditions and the reduction of negative effects of drought stress, inoculating *F. ovin*a seeds with *Azotobacter* and *Pseudomonas* is recommended to improve their growth and development characteristics under drought conditions. PGPR, as an affordable and environmentally friendly method, can improve the production of forage in water-stress rangelands.

Keywords: bio-fertilizers; element uptake; drought stress; rangeland; water scarcity

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1 Introduction

The world faces several notable challenges, i.e., population growth, increasing demand for food, and a wide range of environmental crises. These problems are related to crops and should be dealt (Sarikhani and Amini, 2020; Sofi et al., 2021). Supplying the world food for population is a huge challenge faced by farmers. Furthermore, plant growth is strongly influenced by environmental factors. If an environmental factor is less than ideal, it will limit plant growth and/or distribution (Goswami et al., 2016). For example, only plants that are adapted to limited water can live in the desert. Directly or indirectly, most plant problems are caused by environmental stress. In some cases, poor environmental conditions (e.g., limited water) directly harm the plants. In other cases,

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environmental stress weakens the plant, and makes it more susceptible to disease or insect attack (Sofi et al., 2021). Environmental factors that affect plant growth include light, temperature, aridity, humidity, and nutrients (Brodersen et al., 2019; Moghbeli et al., 2021; Seleiman et al., 2021).

Environmental stresses, particularly aridity, are the most important variables hindering several development phases of plant, most notably seed germination, in arid and semi-arid areas (Devincentis, 2020; Seleiman et al., 2021). Aridity has a number of detrimental effects on the growth of plants, hinders seed germination, and reduces the number of aerial organs and production in crops and plants in rangelands (Diatta et al., 2020). Increased aridity strongly reduces photosynthesis and impairs plant physiological process, ultimately causing plant death. In such situations, selecting the species prone to environmental stresses such as drought, especially at seed germination stage, is vital for harvesting plants in highly water-stressed areas (Wang et al., 2003; Delshadi et al., 2017).

Iran's rangelands are rich in genetic resources for their diversity (Moghbeli et al., 2021) *Festuca ovina* L. is a perennial herb of Poaceae family (Wilkinson and Stace, 1991). This plant is one of Iran's most valuable and popular semi-steppe and mountainous species with an undeniable value in livestock's nutrition and soil conservation (Ghorbani et al., 2013). Cultivating these plants is a great option to produce fodder due to their high quality and aridity resistance. Nevertheless, seeds often fail to germinate in natural conditions. In semi-steppe and mountainous areas, the seed germination of *F. ovina* is possible in suitable conditions and without environmental stresses such as aridity. Therefore, the preservation and development of this species can have a highly influence on improving livestock's production capacity (Ghorbani et al., 2013).

A variety of techniques have been investigated to help plants cope with the negative consequences of drought stress, including inoculation with bio-fertilizers (Arun et al., 2020; Batool et al., 2020). Bio-fertilizers do not exclusively refer to organic matters derived from livestock, plants, and green fertilizers; rather, bacteria and fungi are among the most critical bio-fertilizers. Plant growth promoting bacteria (PGPB) are the scientific name for these bacteria because of their positive impact on plant growth and development. They are used extensively to alleviate biological stress (Delshadi et al., 2017; Batool et al., 2020). The use of PGPB in agricultural production and environmental adaptation can give environmentally benign and long-term solutions. Plant nutrient absorption is improved, soil-borne diseases are reduced, and biological control agents are employed with PGPB (Sheteiwy et al., 2021). They produce a vast variety of enzymes and metabolites. Additionally, they impact nutritional levels, maintain hormonal balance, and minimize the negative consequences of biological and bio-system stresses (Delshadi et al., 2017). Applying PGPR is an attractive strategy to mitigate drought stress (Niu et al., 2018). *Azotobacter* and *Pseudomonas* are two more important genera that have been widely used in plants. *Azotobacter vinelandii* strain O4 can fix air nitrogen and solubilize phosphate (Bianco and Defez, 2011). This bacterium is used as bio-fertilizer to increase plant growth (Nosrati et al., 2014). Also, researches have shown that *Azotobacter* can prevent the osmotic shock of the host plant due to chemical fertilizers (Delshadi et al., 2017) by establishing a symbiotic relationship (Bianco and Defez, 2011; Nosrati et al., 2014). The phosphorus cycle and solubilization of phosphorus in biological cells are both dependent on *Pantoea agglomerans* strain P5 and *Pseudomonas putida* strain P13 (Chen et al., 2015).

Due to drought stress and impending difficulty in accessing water for agriculture purposes, biological treatments can not only improve soil biological activity, but also reduce water consumption, and protect environment from chemical fertilizers, thus increasing the quantity and quality of crops. The study aimed to examine the impacts of applying PGPR on reducing drought stress in *F. ovina*. In this study, it was hypothesized that (1) *A. vinelandii* and *P. agglomerans*+*P. putida* equally reduce the impacts of drought in *F. ovina*; and (2) under drought stress conditions, combined treatments are more effective than separate treatments.

2 Materials and methods

2.1 Preparing culture substrate

To investigate the mutualistic impacts of bio-fertilizers (four levels) and drought regimes (four levels), we made greenhouse culture in a factorial design with three replications and two variables. Bio-fertilizer treatments included *A. vinelandii* (A), the combination of *P. agglomeran* and *P. putida* (S), combined application of *Azotobacter* and *Pseudomonas* (AS), and control treatment (no inoculation). The *Azotobacter* was inoculated in powder form contained 1×10^8 bacteria cell/g. *Pseudomonas* was inoculated in powder form with an approximate population of 1×10^8 bacteria cell/g. Bio-fertilizers were obtained from Sabz Biotechnology Company, Iran. For each bacterial strain, a 250-mL Erlenmeyer flask containing 100 mL nutrient broth was prepared. To prepare the inoculum, we removed a pure colony of each bacterium and added it to an Erlenmeyer flask containing the culture medium under sterile conditions. Erlenmeyer flask inoculated with each bacterium was placed on a shaker at a speed of 120 r/m and a temperature of 28°C. After 48 h of inoculation, the strains with an approximate population of 1×10^8 bacteria cell/g (based on the McFarland standard) were ready for use (Delshadi et al., 2017). Both bio-fertilizers were used in mixed treatments. In single treatment, only one bio-fertilizer (*Azetobacter* or *Pseudomonas*) was used. No bio-fertilizer was used in control treatment. The applied drought regimes included field capacity (FC) (100% FC), 50% FC, 30% FC, and 7% FC. Greenhouse cultivation was carried out in the Research Center for Agriculture and Natural Resources in Kerman, Iran, at temperatures ranging from 12°C to 36°C.

Table 1 shows the properties of the soil used for plant cultivation. The soil was sieved via a 4-mm sieve prior to plant cultivation. First, a very thin fabric was placed over the pots' bottoms to prevent bio-fertilizers from leaching out. A total of 2 kg of soil was then added to each pot. The pots were then irrigated till saturation. Each pot included twenty seeds (at a depth of 0.5 cm). The pots were irrigated daily prior to seed germination. For inoculation, half a gram of each bio-fertilizer was dissolved in water. The seeds were coated in the provided solution (Delshadi et al., 2017). The FC of the soil was first determined using the weighting method before applying drought stress (Diallo and Marico, 2013). Then, we determined the required water amount to apply drought stress based on the calculated FC (Zarik et al., 2016). To that end, first, 2 kg of dry soil of each pot were saturated after weighing (W_1; g) so that the additional water would be extracted by gravity. A thin plastic covering was applied to the posts to prevent evaporation. Then, there were left in the greenhouse under optimum conditions for 48 h so that the additional water would be extracted (Azizi et al., 2020). Then, the saturated soil of each pot was weighed (W_2; g). The weight difference between W_1 and W_2 is the amount of saturation, which, here, was considered as FC value (100%). The water required for each irrigation time per pot was calculated per stress level using the following equations (Azizi et al., 2020):

$$30\%\ \mathrm{FC}=0.3\times(W_2-W_1)\times100\%, \quad (1)$$

$$50\%\ \mathrm{FC}=0.5\times(W_2-W_1)\times100\%, \quad (2)$$

$$70\%\ \mathrm{FC}=0.7\times(W_2-W_1)\times100\%. \quad (3)$$

Maguire's formula (1962) was used to calculate germination percentage (GP) and rate (GR). In other words, the number of seeds that germinated in each pot was used to compute the total number of seeds that germinated, and GR was estimated by Equation 4:

$$\mathrm{GR}=\sum N_i D_i, \quad (4)$$

where GR, N_i, and D_i indicate the germination rate (numbers/d), the number of germinated seeds,

Table 1 Soil characteristics used for plant cultivation

Soil texture	EC (dS/m)	pH	TN (%)	TP (mg/kg)	SOM (%)	K (mg/kg)
Loamy	0.21	5.30	0.20	18.34	1.94	630

Note: EC, electrical conductivity; TN, total nitrogen; TP, total phosphorus; SOM, soil organic matter; K, potassium.

and the number of days following planting, respectively. After 14 d, the plants thinned. Ten plants remained in each pot. The species were harvested after 90 d. To remove rhizosphere soil from seedling roots, we rinsed them with distilled water. After that, a ruler was used to determine the lengths of stems and roots. Following that, the samples were dried for 48 h at 72°C to determine the dry weight of plant biomass using a 0.001 precision digital scale.

2.2 Measuring absorption of nutrients

Nitrogen (N), phosphorus (P), potassium (K), zinc (Zn), iron (Fe), and manganese (Mn) concentrations in tissues were determined. First, plant essence was extracted using the wet digestion method (Rayan et al., 2001). The amounts of N, P, and K were respectively measured by the titration method, chlorometric analysis method (spectrophotometer), and flame atomic emission spectroscopy (Rayan et al., 2001). Zn, Fe, and Mn concentrations were determined by Inductively Coupled Plasma-Optical Emission Spectrometer (ICP-OES, Vista AX and RL, Varian, Mulgrave, Australia).

2.3 Statistical analysis

The one-way analysis of variance (ANOVA) method was used for the statistical analysis of the data. Prior to analysis, the Kolmogorov–Smirnov test was used to determine normality, and the Levene's test was used to determine variance homogeneity (P<0.05), and data subsequently log-transformed as required. Significance was defined as a probability of 0.05. SPSS v.20.0 was used to conduct all statistical calculation.

3 Results

3.1 Effect of bio-fertilizers on seed germination

The data analysis revealed that drought stress, bio-fertilizers, and their interaction had a significant effect on seed germination (P<0.01). Comparing the means of the applied drought indicated that GR and GP decreased significantly by increasing aridity (P<0.01). The 30% FC stress resulted in the lowest GP. The stress-free treatment displayed the highest GP and GR (Table 2). As shown in Table 2, the application of *Azotobacter* and *Pseudomonas* in stress-free conditions indicated that both bio-fertilizers had a significant positive impact on seed germination (P<0.01). However, the combined application of bio-fertilizers (AS) was more effective than applying separate treatments. AS treatment displayed the maximum seed germination (GR=26.59 numbers/d, GP=100.00%) in stress-free conditions.

The interaction of drought and separate application of *Azotobacter* (Table 2) indicated that the maximum GP and GR significantly occurred in 50% FC treatment (P<0.01) (GR=24.60 numbers/d, GP=96.97%). The separate application of *Pseudomonas* yielded similar results. The maximum GP was observed in 50% FC treatment. Increasing stress from 50% FC to 30% FC and the separate application of both bio-fertilizers significantly reduced seed germination (P<0.01), indicating the diminishing efficiency of *Azotobacter* and *Pseudomonas* under drought stress. Compared with separate treatments (A) and (S), the combined treatment (AS) and different drought regimes had more positive effects on seed germination: the germination increased significantly as stress increased from 70% FC to 30% FC (P<0.01). The treatments including AS+100% FC, AS+70% FC, and AS+50% FC did not display a significant (P>0.01) effect on seed germination.

3.2 Effect of bio-fertilizers on plant growth

The data analysis of plant characteristics indicated significant effect of drought and bio-fertilizers, as well as their interaction effects on the roots and stem lengths, and the dry weight of aerial organs and roots (P<0.05). Data comparison showed that drought stress (Table 3) resulted in a reduction in the stem and root lengths, as well as the plant's dry weight. The longitudinal growth and dry weight of roots and aerial organs significantly decreased (P<0.05), as the stress increased from 70% FC to 30% FC. Treatment 30% FC had the least growth rate.

Table 2 Drought and bio-fertilizers effects on GR and GP of *Festuca ovina*

Treatment	GR (numbers/d)	GP (%)
100% FC	20.20±0.60^{a}	87.29±4.15^{a}
70% FC	18.22±0.60^{b}	80.39±4.15^{b}
50% FC	15.15±0.50^{c}	75.12±3.25^{c}
30% FC	12.21±0.50^{d}	60.12±3.65^{d}
A	24.22±0.70^{b}	95.63±5.00^{b}
S	24.09±0.70^{b}	95.33±5.00^{b}
AS	26.59±0.71^{a}	100.00±5.12^{a}
N'	18.92±0.50^{c}	89.00±4.00^{c}
A+100% FC	23.13±0.70^{b}	95.77±4.90^{a}
A+70% FC	24.53±0.72^{a}	96.88±4.90^{a}
A+50% FC	24.60±0.72^{a}	96.97±4.90^{a}
A+30% FC	18.21±0.60^{c}	83.13±3.44^{b}
S+100% FC	22.00±0.72^{b}	95.12±5.00^{a}
S+70% FC	24.20±0.72^{a}	96.13±5.00^{a}
S+50% FC	24.07±0.70^{a}	97.65±5.23^{a}
S+30% FC	17.71±0.50^{c}	81.32±4.17^{b}
AS+100% FC	24.67±0.80^{b}	96.00±6.50^{b}
AS+70% FC	24.86±0.80^{ab}	97.40±5.00^{b}
AS+50% FC	24.89±0.80^{ab}	97.89±5.00^{b}
AS+30% FC	25.16±0.80^{a}	99.62±3.40^{a}
N'+100% FC	19.10±0.50^{a}	85.10±4.80^{a}
N'+70% FC	17.11±0.40^{b}	79.10±4.50^{b}
N'+50% FC	14.06±0.40^{c}	74.12±4.50^{b}
N'+30% FC	13.12±0.40^{c}	62.10±4.50^{c}

Note: Different lowercase letters within the same column indicate significant difference among treatments at P<0.01 level. Mean±SE. GR, germination rate; GP, germination percentage; A, *A. vinelandii*, S, *P. agglomerans*+*P. putida*; AS, *A. vinelandii*+*P. agglomerans*+*P. putida*; FC, field capacity; N', no inoculation. The abbreviations are the same in the following table.

The main effects of *Azotobacter* and *Pseudomonas* on plant growth (Table 3) indicated that both fertilizers considerably increased plant growth (P<0.05). Similar to seed germination results, the combined AS treatment was more effective than applying them separately. AS treatment exhibited the highest plant growth indices under drought stress, with a notable difference (P<0.05) from the other treatments.

The interaction results of different drought stress and separate application of *Azotobacter* (Table 3) indicated that, in drought conditions, *Azotobacter* increased plant growth rate. Compared with A+100% FC treatment, as stress increased from 70% FC to 50% FC, plant growth increased significantly (P<0.05). The highest longitudinal growth of aerial organs and roots was observed in A+50% FC treatment. The A+30% FC treatment displayed a significant plant growth (P<0.05), indicating the inefficiency of *Azotobacter* to improve plant growth in 30% FC treatment. The mutual interaction of *Pseudomonas* and drought stress (Table 3) showed that in the presence of *Pseudomonas*, as drought stress increased, plant growth increased. The maximum growth index studied were found in S+30% FC treatment. Although increasing the stress concentration from 70% FC to 30% FC did not result in a significant increase (P>0.05) in plant growth rate.

The interaction of AS treatments under droguht stress indicated the considerable performance of combined treatments compared with separate applications of *Azotobacter* and *Pseudomonas* (Table 3). With the transition from 100% FC to 30% FC conditions, the length of the roots and stems and the biomass of aerial organs and roots increased significantly (P<0.05). The largest

growth of the plant was observed in AS+30% FC treatment, displaying significant difference (P<0.05) from the other treatments.

Table 3 Morphological traits of *Festuca ovina* under drought stress and bio-fertilizers

Treatment	Root length (cm)	Stem length (cm)	Root dry weight (mg)	Aerial dry weight (mg)
100% FC	9.09±0.30^{a}	11.33±0.40^{a}	0.92±0.00^{a}	1.90±0.00^{a}
70% FC	7.11±0.30^{b}	9.80±0.40^{b}	0.82±0.00^{b}	1.80±0.00^{b}
50% FC	5.18±0.20^{c}	7.40±0.40^{c}	0.65±0.06^{c}	1.65±0.00^{c}
30% FC	4.23±0.10^{d}	5.27±0.20^{d}	0.50±0.00^{d}	0.92±0.00^{d}
A	11.33±0.30^{b}	13.29±0.51^{b}	0.67±0.02^{b}	2.67±0.02^{b}
S	11.62±0.30^{b}	13.87±0.51^{b}	0.75±0.02^{ab}	2.75±0.02^{ab}
AS	13.10±0.30^{a}	15.34±0.60^{a}	1.55±0.02^{a}	3.24±0.02^{a}
N'	9.21±0.30^{c}	11.14±0.40^{c}	1.42±0.02^{b}	1.23±0.01^{b}
A+100% FC	11.13±0.41^{c}	13.40±0.60^{c}	0.73±0.01^{c}	2.25±0.02^{c}
A+70% FC	12.44±0.41^{b}	14.85±0.60^{b}	0.89±0.01^{b}	2.60±0.02^{b}
A+50% FC	13.32±0.41^{a}	15.60±0.60^{a}	0.96±0.01^{a}	2.89±0.02^{a}
A+30% FC	8.22±0.30^{d}	10.57±0.40^{d}	0.64±0.01^{d}	0.92±0.00^{d}
S+100% FC	11.24±0.45^{b}	13.92±0.60^{b}	0.84±0.00^{b}	2.30±0.03^{b}
S+70% FC	12.62±0.45^{a}	14.11±0.60^{a}	1.23±0.02^{a}	2.69±0.03^{a}
S+50% FC	12.96±0.55^{a}	14.73±0.60^{a}	1.32±0.02^{a}	2.83±0.03^{a}
S+30% FC	12.67±0.55^{a}	14.80±0.60^{a}	1.41±0.02^{a}	2.96±0.03^{a}
AS+100% FC	13.24±0.55^{d}	15.96±0.60^{d}	2.46±0.03^{d}	3.56±0.03^{d}
AS+70% FC	14.35±0.60^{c}	16.92±0.62^{c}	2.65±0.03^{c}	3.77±0.03^{c}
AS+50% FC	15.76±0.60^{b}	17.86±0.62^{b}	3.20±0.03^{b}	4.50±0.03^{b}
AS+30% FC	17.43±0.60^{a}	19.88±0.70^{a}	3.86±0.03^{a}	4.96±0.03^{a}
N'+100% FC	9.66±0.45^{a}	11.43±0.40^{a}	0.89±0.01^{a}	1.65±0.03^{a}
N'+70% FC	7.42±0.45^{b}	9.31±0.40^{b}	0.76±0.01^{b}	0.93±0.03^{b}
N'+50% FC	6.76±0.35^{c}	8.10±0.40^{c}	0.56±0.01^{c}	0.76±0.00^{c}
N'+30% FC	6.12±0.35^{c}	7.14±0.40^{d}	0.43±0.01^{c}	0.61±0.00^{c}

Note: Different lowercase letters within the same column indicate significant difference among treatments at P<0.01 level. Mean±SE.

3.3 Effect of bio-fertilizers on element uptake

Data analysis indicated that drought stress, bio-fertilizers, and their interaction showed significant impacts on element uptake (P<0.05). Comparing the data indicated that the increased drought stress (Figs. 1, 2, and 3) reduced the quantities of micro and macro elements in aerial organs. The 70% FC stress did not have any significant impact on the quantities of these elements (P>0.05). As the stress increased from 70% FC to 30% FC, the absorption of all elements substantially decreased (P<0.01). The lowest amount of absorption of elements was measured in 30% FC treatment (Figs. 1a, 2a, and 3a).

Comparing the average impact of bio-fertilizers revealed that treatment A (P<0.05) had the highest significant concentrations of N (Fig. 1b), K (Fig. 2b), and Fe (Fig. 3b). The maximum concentrations of P (Fig. 1b) and Zn (Fig. 3b) were observed in S treatment. The highest concentration (P<0.05) of Mn was found in the combined AS treatments (Fig. 2b).

The interaction effects of drought and separate application of *Azotobacter* in the absorption of N, P (Fig. 1c), and K (Fig. 2c) showed that in drought conditions, *Azotobacter* significantly increased the quantities of these elements (P<0.05). Compared with FC, increasing stress from 70% FC to 30% FC significantly increased the quantities of these elements (P<0.05). *Azotobacter* did not significantly impact the absorption of Mn in drought stress (Fig. 2c). The highest amount of Mn was measured in A+100% FC treatment (Fig. 2c). The transition of stress from 100% FC to

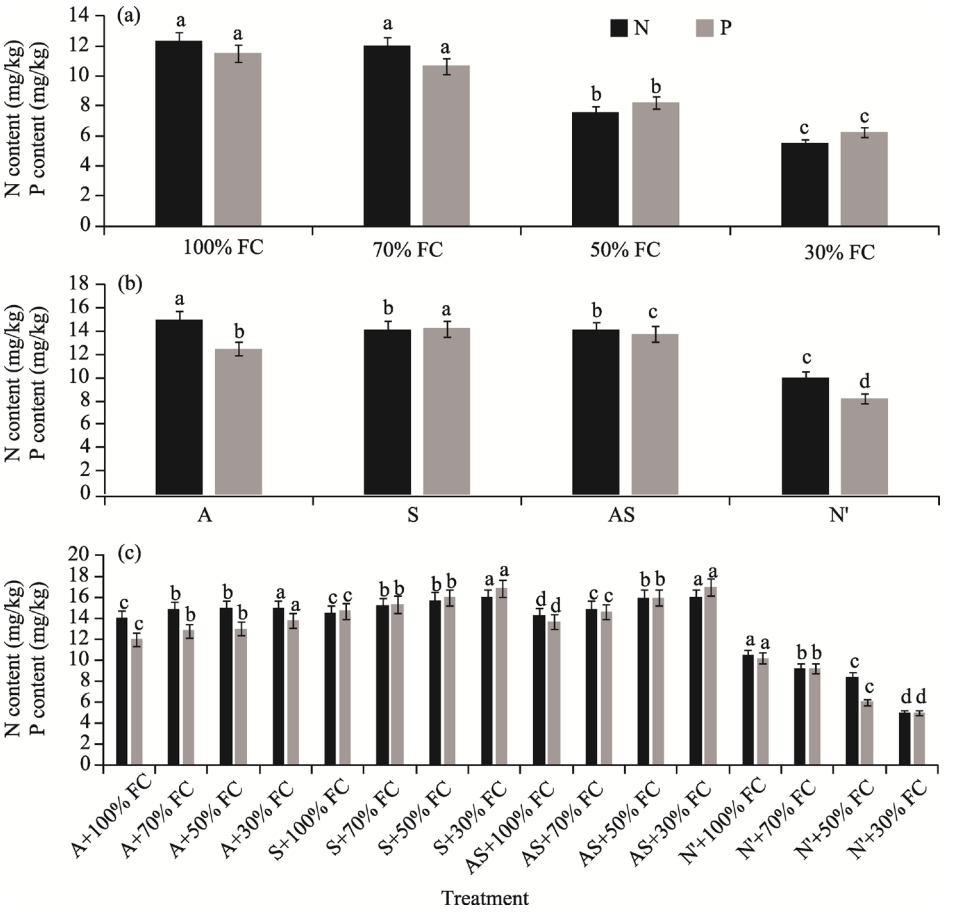

Fig. 1 Impact of drought stress (a), bio-fertilizers (b), and interaction effect between bio-fertilizers and drought stress (c) on absorption of nitrogen (N) and phosphorous (P). Bars are standard errors. A, *A. vinelandii*; S, *P. agglomerans*+*P. putida*; AS, *A. vinelandii*+*P. agglomerans*+*P. putida*; N', no inoculation; FC, field capacity. Different lowercase letters within different treatments in Figure 1a and b indicate significant differences at $P<0.05$ level for N or P. Different lowercase letters within the same bacterial and different FC treatments in Figure 1c indicate significant differences at $P<0.05$ level for N or P.

50% FC increased Fe (Fig. 3c) and Zn (Fig. 3c) absorption significantly in the presence of *Azotobacter* ($P<0.05$). However, in 30% FC treatment, the quantities of Zn and Fe decreased significantly (Fig. 3c).

The interaction between *Pseudomonas* and drought stress demonstrated that when drought stress increased, the amounts of N (Fig. 1c), P (Fig. 1c), K (Fig. 2c), and Zn (Fig. 3c) were substantially enhanced in the presence of *Pseudomonas* compared with bio-fertilizer free treatment ($P<0.05$). The maximum quantities of these elements were observed in S+30% FC treatment. As stress increased from 100% FC to 30% FC, the absorption of Mn decreased significantly ($P<0.05$), indicating the ineffectiveness of *Pseudomonas* in improving the absorption of Mn in drought conditions (Fig. 2c).

The combined AS treatment in drought stress had the highest impact on the absorption of all test elements. It also displayed a higher nutrition absorption efficiency than the separate application A and S treatments. From 100% FC to 30% FC, the absorption of all elements increased significantly ($P<0.05$). The highest quantities of elements were observed in AS+30% FC treatment, indicating a significant ($P<0.05$) difference from the other treatments.

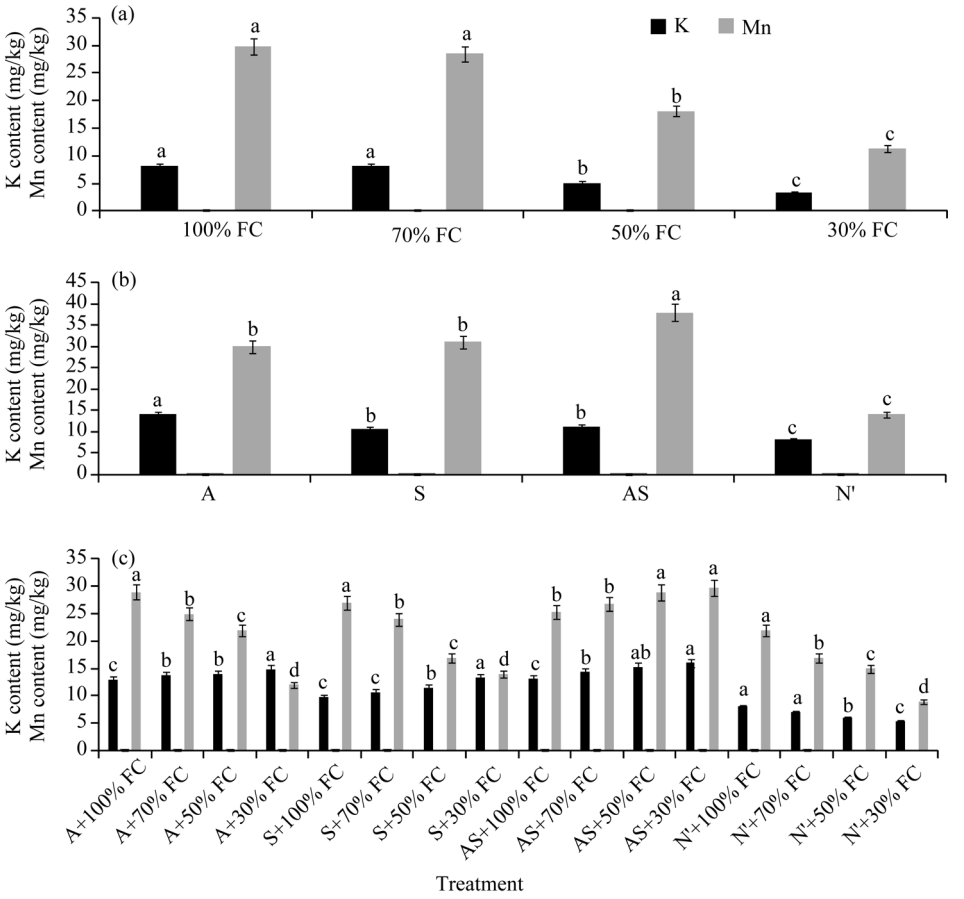

Fig. 2 Impact of drought stress (a), bio-fertilizers (b), and interaction effect between bio-fertilizers and drought stress (c) on absorption of potassium (K) and manganese (Mn). Bars are standard errors. A, *A. vinelandii*; S, *P. agglomerans*+*P. putida*; AS, *A. vinelandii*+*P. agglomerans*+*P. putida*; N', no inoculation; FC, field capacity. Different lowercase letters within different treatments in Figure 2a and b indicate significant differences at $P<0.05$ level for K or Mn. Different lowercase letters within the same bacterial and different FC treatments in Figure 2c indicate significant differences at $P<0.05$ level for K or Mn.

4 Discussion

4.1 Seed germination

The results indicated that an increase in stress from 70% FC to 30% FC reduced seed germination. The various studies on different plants indicated that by increasing osmotic stress, including drought stress, the percentage of germination and biomass of plant organs decrease (Armand et al., 2015). This decrease is due to the increased concentration of polyethylene glycol, reducing the absorption of water by seeds, and thwarting the natural activities of the plant. Drought reduces seed germination by delaying the absorption of water by seeds (Liu et al., 2015). Drought reduces water absorption, thus reducing enzyme activities related to the biochemical processes of seed germination (Zamani et al., 2018). At high drought stress levels, the potential damages due to the changes in enzyme structure can be one of the main reasons for the decrease in germination rate (Fabian et al., 2008). Besides, if the absorption of water by the seed is disrupted or delayed, it leads to decelerated physiological and metabolic processes inside the seed. This prolongs the time necessary for the steckling to escape the root and reduces the rate of seed germination (Fig. 4). Reduced seed germination is practically an adaptive solution that allows the plant to wait for proper conditions (Sabeti et al., 2019).

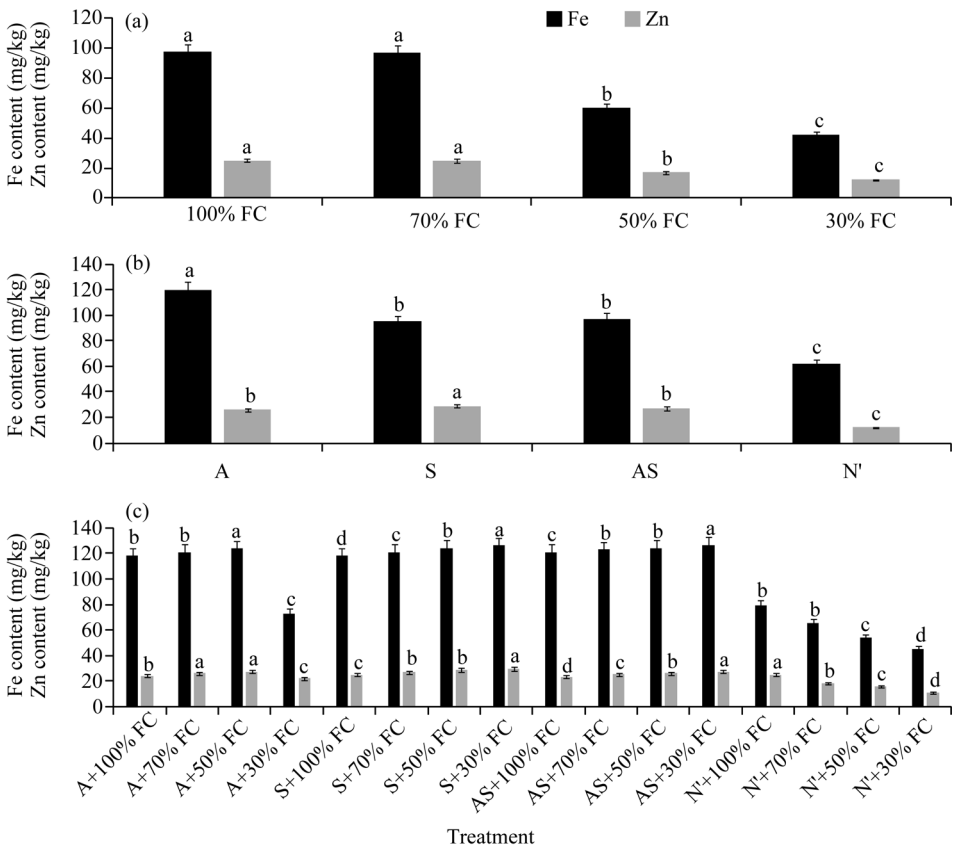

Fig. 3 Impact of drought stress (a), bio-fertilizers (b), interaction effect between bio-fertilizers and drought stress (c) on absorption of iron (Fe) and zinc (Zn). Bars are standard errors. A, *A. vinelandii*; S, *P. agglomerans*+*P. putida*; AS, *A. vinelandii*+*P. agglomerans*+*P. putida*; N', no inoculation; FC, field capacity. Different lowercase letters within different treatments in Figure 3a and b indicate significant differences at $P<0.05$ level for Fe or Zn. Different lowercase letters within the same bacterial and different FC treatments in Figure 3c indicate significant differences at $P<0.05$ level for Fe or Zn.

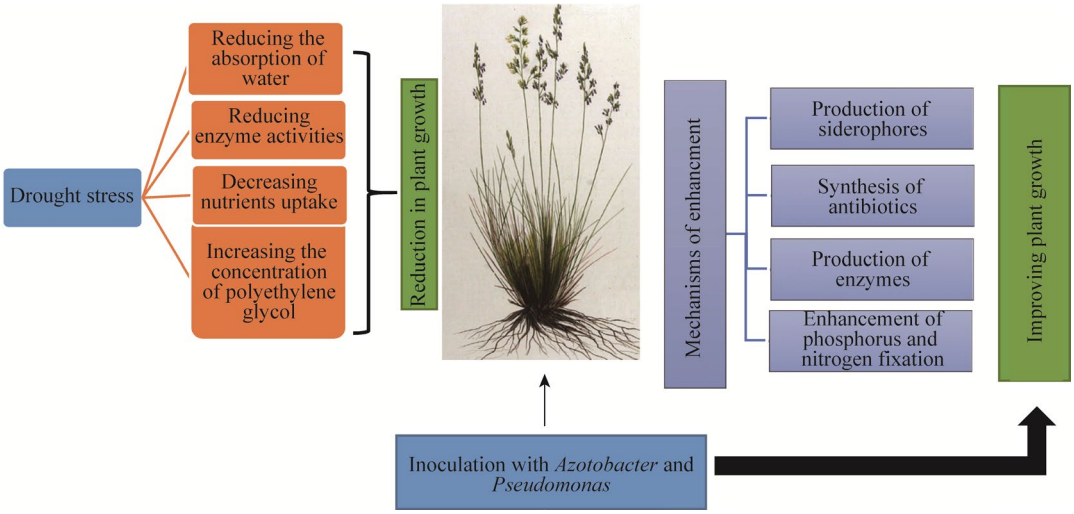

Fig 4 Mechanism of plant growth-promoting bacteria under drought stress

The application of A and S treatments in this study indicated that both bio-fertilizers significantly increased seed germination in both stress-free and drought stress conditions.

Although in 30% FC condition seed germination decreased, the applied bio-fertilizers had a positive effect on seed germination. Researchers have attributed this result to the high absorption of water and the higher activity of alpha-amylase in the presence of these microorganisms (Batool et al., 2020). Alpha-amylase is one of the vital enzymes in the metabolism of carbohydrates in the seed germination process that hydrolyzes polysaccharides such as starch (Delshadi et al., 2017; Batool et al., 2020). This enzyme plays an essential role in germination and breaking starch into simple sugars. Enzyme activity is more applicable in germinating seeds as a catalyst to improve seed germination. It has been suggested that multiple strains of *Pseudomonas* and *Azotobacter* enhance plant growth via a variety of mechanisms, including the production of siderophores, the synthesis of antibiotics, the enhancement of P and N fixation, and the production of enzymes that regulate the concentration of ethylene (Abdul-Jaleel et al., 2007; Abbassi and Koocheki, 2008; Sabeti et al., 2019).

To overcome the detrimental impacts of drought and improve plant development, researchers required comprehensive solutions. According to Sheteiwy et al. (2021), drought stress increased bacterial counts, which might be due to soil drying and rewetting, could produce a major shift in the constitution of organic matter and its components, making it more susceptible to microbial activity. Furthermore, during drought stress, microorganisms may enter latent phases, allowing bacteria to quickly degrade organic molecules upon rewetting.

It's believed that PGPB seed inoculation might lead to the production of indole acetic acid (IAA), an important stimulant of plant cell growth and cell lengthening that can help seeds germinate more quickly in response to the amino acids released by the seed (Barnawal et al., 2017; Lu et al., 2018). Seed priming with bio-fertilizers also reduced seed exposure to drought stress, induced radicle emergence, and decreased adverse effects of stressful factors (Sabeti et al., 2019). Omara and Elbagory (2018) also observed that when wheat seedlings were exposed to drought stress, PGPB improved seed germination (*Triticum aestivum* L.). The result confirmed that seed inoculation with PGPB accelerated the release of enzymes involved in seed germination, and increased seed germination compared with non-inoculation conditions.

According to the data, combined treatment of AS had more effects on reducing drought stress on seed germination than single application of bio-fertilizers. Delshadi (2015) stated that although inoculation of seeds with only a specific strain of bio-fertilizers could increase plant yield by absorbing nutrients and promoting plant yield due to the secretion of secondary metabolites, sometimes their combined use enhances their effects and leads to increased plant yield and plant tolerance to stress.

4.2 Plant morphological traits

The results indicated that with increasing drought stress, plant growth indices decreased. Reduced cell growth inhibits organ growth. The first noticeable effect of water shortage on plants can be traced to reduced height (Bechtold, 2018). Stopping growth during stress conditions largely depends on stress intensity. The intense osmotic stress immediately stops the growth of leaves and stem (Sehgal et al., 2018). In their study of the relationships between spectral properties and yield and nutritional quality of *Phaseolus vulgaris* L. in dry years, Nemeskéri et al. (2018a) discovered that plant height was reduced, and the leaf area index was low in non-irrigated circumstances.

Some researchers stated that reducing water potential caused by drought stress could change plant morphology and physiology via disturbing ionic balance, intensifying toxic ions, disrupting physiological and metabolic processes, including absorption, transport, reduction, and metabolism of N and protein, closing stomata, degrading cell membrane, decreasing cell division, reducing photosynthetic efficiency, and increasing metabolite toxicity (Zawoznik et al., 2011; Rojas-Tapias et al., 2012). To conserve water, plants reduce their stomatal conductance. As a result, CO_2 fixation and photosynthetic rate decrease (Mafakheri et al., 2010; Delshadi et al., 2017), leading to lower assimilate production for plant yield (Nemeskéri et al., 2018b). Under drought conditions, diffuse resistance of the stomata to CO_2 entry is most likely the most important factor limiting photosynthesis (Boyer, 1970). Water scarcity also reduces plant

photosynthesis by changing chlorophyll concentration, damaging chlorophyll particles, and harming photosynthetic system (Mafakheri et al., 2010; Nemeskéri et al., 2019). According to Nemeskéri and Helyes (2019), during dry periods, tomatoes grown in non-irrigated conditions had low photosynthetic activity, but under moderate and optimal water supply conditions, photosynthetic activity was higher. Nemeskéri et al. (2018b) discovered that non-irrigated conditions had the highest stomatal tolerance of leaves and the lowest leaf area index and harvest index for the pods of snap beans.

Drought stress reduces plant growth phytohormones, e.g., gibberellic (GA) and auxin, and increases growth inhibitors, especially abscisic acid (ABA), by affecting plant hormonal balance (Andersen et al., 2002). Phytohormones have been extensively researched in the management of plant and seed growth in response to environmental stress. Despite the fact that numerous studies have been conducted in the last decade to better understand their function during seed maturation and ripening, experimental support for their function, biosynthetic route, and signal transmission during seed development under drought stress is lacking (Sheteiwy et al., 2021). ABA is a well-known hormone that impacts seed development and growth by enhancing seed protein synthesis and changing seed fullness (Li et al., 2018; Sheteiwy et al., 2021). Additionally, ABA has a role in the accumulation of fatty acids (FA) during seed maturation. This analysis discovered low ABA levels and elevated GA, IAA, and GA levels. Sheteiwy et al. (2021) highlighted how inoculating soybean with *Bacillus amyloliquefaciens* and mycorrhiza resulted in low ABA levels but high GA and IAA levels.

Reducing indices of fresh and dry weight in plants under drought conditions has been widely confirmed in other studies, which was attributed to osmotic pressure in the root and reduced some characteristics, such as water uptake, growth, and finally plant weight (Emadi et al., 2009). Drought stress, apart from decreasing capacity for carbon storage and limiting leaf gas exchange, also affects the transport of photosynthetic materials that can lead to saturating leaves with these compounds, limiting the photosynthesis process, and reducing plant biomass. Thus, the decrease in photosynthesis observed in plants under adverse climatic circumstances, particularly drought stress, might be attributable to changes in chlorophyll concentration, damage to the photosynthetic system, or a combination of these two (Nadeem et al., 2014).

Plant growth increased as drought stress increased in the presence of *Azotobacter* and *Pseudomonas*. Although S treatment increased plant growth under 30% FC stress, compared with A treatment, the combined AS treatment was more effective against drought compared with the separate application of treatments. Numerous studies have been undertaken to determine the efficacy of PGPB in improving plant growth under drought-stressed conditions. It is widely established that PGPB are excellent in enhancing the development of a wide variety of plants under stressful circumstances, including legumes, cereals, and vegetables (Sandhya et al., 2010). Abiotic stresses may be mitigated by using PGPB, which increase the availability of nutrients for plants (Ngumbi and Kloepper, 2016). It has been established that rhizobacteria may create exopolysaccharides (EPS) including alginate and cellulose, which can improve drought tolerance (Zahir et al., 2009), making PGPB useful for enhancing plant development under drought conditions (Nadeem et al., 2010). As a result, EPS may play an important role in alleviating the consequences of drought on plants. EPS that form the attachment space among bacteria and root systems, soil particles, and other bacteria (Yang et al., 2016). PGPB create EPS, which might aid plant development under drought stress by providing a barrier surrounding the roots (Abdelaal et al., 2021). Moreover, flavonoids found in the rhizome of PGPB also induce lipo-chitooligosaccharides, which are excreted by PGPB (Gepstein and Glick, 2013). Trace amounts of the non-reducing sugar trehalose are vital for improving plant tolerance to a wide range of abiotic stress, including drought, which is the reason for its importance for cultivating crops that contain trehalose. It is a relatively stable molecule, and it may help minimize drought damage by controlling the accumulation and breakdown of proteins that happen in a variety of conditions (Jalili et al., 2009).

According to some studies, plants that have been exposed to drought stress have higher growth

rates because of the increased production of enzyme 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase, which increases the absorption of nutrients, including N, P, and K. This beneficial impact may be a result of increased water usage efficiency and increased antioxidant enzymatic activity in response to drought stress. As a consequence, PGPB promote growth characteristics by releasing hormones, such as IAA, GA, and cytokinins, which results in increased nitrification and enhanced absorption of nutrients. Moreover, PGPB play a critical function in reducing drought stress response caused by the formation of ABA. Root growth may benefit from the antioxidant properties of PGPB (Garcia de Salamone et al., 2001). Additionally, proline and various mineral nutrients (osmolytes) accumulation may also be a means through which PGPB benefit plants like *Cichorium pumilum* Jacq. (Saikia et al., 2018). During drought conditions, PGPB may directly or indirectly promote ACC deaminase activity, hence improving plant function (Sarabi and Arjmand-Ghajur, 2021). A plant-derived ACC oxidase first absorbs and then degrades ACC in this approach. As a result of their ability to reduce ethylene concentrations, PGPB may serve as an excellent provider of growth factors and osmotic stress (Santoyo et al., 2016). Seed inoculation increased plant height and root length in cotton, wheat, and maize (Narula et al., 2005), indicating the favorable effects of PGPB on plant morphology. Other studies have shown that PGPB improve pistachio seedling development and nutrient intake, as well as rice and wheat growth indices, when grown under drought stress conditions (Sarcheshmehpour et al., 2013; Rana et al., 2015).

4.3 Plant nutrients uptake

N is an essential element for plant growth, accelerates growth, and increases crop yield. K facilitates water penetration into plant cells, and controls the opening and closing of leaf stomas during evaporation and transpiration. Absorbing N, P, protein, and starch synthesis are among the roles of K. P plays an important role in the process of photosynthesis. It is especially important in seed germination, root growth acceleration, seed and fruit ripening processes, cell division, and tissue growth (Delshadi et al., 2017; Sofi et al., 2021).

Fe plays an important role in the production of chlorophyll, respiration, and photosynthesis of plants. The absorption of Fe by the plant depends on the pH of soil and its compounds, and this element is often added to the soil in the form of Fe chelate (Anjum et al., 2019). Zn has an activating or structural catalytic role in many plant enzyme systems and is also involved in the construction and breakdown of proteins in plants. Also, Zn is involved in the production of amino acid tryptophan as a prerequisite for the production of auxin hormone, which is effective in the longitudinal growth of branches. The role of Mn in plants is participation in complex systems. Mn is involved in electron transfer reactions in plants and also plays a role in chlorophyll production. Most of Mn was present in the leaves and stems, and its amount was negligible in plant seeds (Anjum et al., 2019; Nedjimi, 2022).

Soil microorganisms play key roles in soil nutrient cycling, structural formation, and plant interactions (Cui, 2021; Gou et al., 2023). Studies show that human activities and environmental factors can directly or indirectly affect the physical-chemical properties of soil (Pang et al., 2019; Gou et al., 2023), and affect the structure and function of soil microbial communities (Zhao et al., 2020; Gou et al., 2023). The change of soil bacterial affects plant productivity as it can lead to regulatory changes in plant nutrient availability (Zhao et al., 2018).

The results showed that bio-fertilizers treatment was more effective in the uptake of elements than control treatment. Increased concentrations of N in wheat (Kizilkaya, 2008), P, and Zn in *Onobrychis sativa* L. (Delshadi et al., 2017), and uptake of macro- and micro-nutrients in wheat (Turan et al., 2012) have also been reported. *Pseudomonas* spp. bacteria can release insoluble P as organic phosphorous acids by lowering soil pH, increasing EC of soil and contributing to enzymatic processes, thereby increasing the mobility of this element in the soil (Delshadi et al., 2017). Additionally, the researchers have discovered an increase in plant uptake of P when P-solubilizing microorganisms coexist in the soil (Rodríguez and Fraga, 1999) and the development of plant roots due to increased uptake of nutrients in bio-fertilizer treatments

(Budania and Yadav, 2014).

The mechanism of absorption and transfer of nutrients in plants, such as mass flow, diffusion or absorption, and transfer by osmotic phenomenon, are all a function of the amount of moisture in the soil and plant roots, and if the moisture decreases, the absorption of nutrients decreases (Karimi et al., 2020). When drought stress occurs, the active absorption of nutrients also decreases, but in this condition, with the stomata closing, the production of photosynthetic materials and their storage in plant tissues decreases more compared with the absorption of nutrients (Jafarian et al., 2012). For this reason, in the condition of drought stress, the content of some nutrients such as P is higher than control treatment. In this study, *Azotobacter* had a significant effect on the absorption of N, Fe, and K, so it can be stated that the use of *Azotobacter* bacteria through improving plant biomass, continuous and stable supply of minerals, especially N, may increase plant growth. In addition, the secretion of hormones such as cytokinin and auxin increases the plant's efficiency.

The results showed that the use of *Pseudomonas* was more effective than the control treatment in absorbing N, Zn, and P by the plant. *Pseudomonas* bacteria can release insoluble P as organic phosphorus acids by increasing soil acidity, increasing soil electrical conductivity, and helping enzymatic processes, and as a result, increase the mobility of this element in the soil (Delshadi et al., 2017). In addition, researchers have reported the increase in P absorption by plants in the coexistence of P-dissolving microorganisms in the soil (Rodríguez and Fraga, 1999) and also the development of plant roots due to the increase in nutrient absorption in bio-fertilizer treatments (Bhatia et al., 2014).

The combined AS treatment had the greatest effect on the uptake of nutrients in 30% FC treatment. The inoculation of the two fertilizers A and S demonstrated a synergistic and intensifying interaction that enhanced microbial biomass and activity to increase mineral intake from the soil, and subsequently improved some plant traits. Combining several kinds of PGPB seems to result in an intensifying interaction, so amplifying the beneficial effects of bacteria, such as enhancing the plant's uptake of water and nutrients from the soil, and thereby promoting plant growth (Delshadi et al., 2017).

5 Conclusions

Our results revealed that drought stress dramatically decreased growth and development stages in *F. ovina*. However, treatment of PGPB mitigated the harmful effects of drought stress and resulted in improved seed germination, growth, and establishment of *F. ovina*. Drought stress during seed germination is associated with a decrease in the water potential and water availability of the plant, and causes a decrease in the indicators of seed germination and plant growth. In the present study, *Azotobacter* and *Pseudomonas* increased germination indices, dry weight, stem length, and root length. In general, inoculation of *F. ovina* seeds with *Azotobacter* and *Pseudomonas* is recommended to improve its growth and development characteristics under drought stress because of the positive effects of PGPB on the growth characteristics of the plant.

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